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NITROGEN METABOLISM AND TROPHIC INPUT IN RELATION TO GROWTH IN FRESHWATER AND SALTWATER *SALMO GAIRDNERI*

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The downstream migration of juvenile members of the *Salmo* genus is occasioned by characteristic morphological, physiological and behavioral alterations, by which stenohaline freshwater parr are transformed into euryhaline migratory smolt (Baggerman, 1960; Hoar, 1963; Fessler and Wagner, 1969), capable of ascending the estuarine salinity gradients to the coastal environments (Koch, Evans and Bergström, 1959; Houston, 1961). This transformation is of a seasonal and transitory nature, maximum euryhalinity coinciding with the onset of the migratory period. Saltwater tolerance is only retained if the seaward migration is completed. Fish that remain in fresh water at the end of the migratory period revert to a near stenohaline condition known as post-smolt until the following spring when they again transform to euryhaline smolt (Koch and Evans, 1962; Conte and Wagner, 1965). In addition, a certain size has to be attained before salmonids can reach their full seasonal hypo-osmotic regularity potential (Conte and Wagner, 1965). This possibly explains the narrow size distribution of 14–18 cm fork-length found in migratory *S. gairdneri* smolts (Mayer and Larkin, 1954; Wagner, Wallace and Campell, 1963).

The size-dependent, as opposed to age-dependent development of euryhalinity has stimulated speculation as to whether growth regulatory factors play a role in the maturation process of the euryhaline ionic regulatory systems in salmonids (Houston and Threadgold, 1963; Parry, 1960). Evidence also suggests continued regulation in an hyperionic environment is concomitant with increased growth rates. Landlocked salmonids are normally of a smaller size at maturity than migratory members of the same species (Ricker, 1938; 1940). This is possibly a direct environmental effect, since offspring of nonmigratory lacustrine parents grow as large as the sea-run type if given the opportunity to migrate to the sea (Foerster, 1947). Furthermore, under both natural and laboratory conditions, saltwater salmonid populations exhibit increased growth rates compared to those in fresh water (Canagaratnam, 1959; Falk, 1969). Such increased growth rates may result from higher food availability, environmental stimulation of food consumption, greater assimilation efficiency of consumed food, or increased retention of assimilated food under saline conditions.

The present study is designed to gain information on the differential growth rates. Specific growth rates and protein metabolism are investigated in relation to trophic input in freshwater smolt and post-smolt and saltwater fish.

Nitrogen balance (Birkett, 1969; Gerkin, 1971) is used to represent protein metabolism and is investigated at the two ration extremes of fully fed and starving

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fish to ascertain which levels of the food conversion process are affected by increased environmental salinity.

Materials and Methods

The experimental fish were yearling, hatchery-reared rainbow trout (*S. gairdneri*) from the Trent Valley Trout Farm, Derby, England. All experimental fish were between 30–40 g and over 15 cm fork-length, the minimum size requirement for euryhaline development during parr-smolt transformation (Conte and Wagner, 1965). The fish were acclimatized to an optimum growth of 12° C temperature (Swift, 1961), and to a day-night cycle of alternating twelve-hour periods for at least four weeks prior to experimentation. The saltwater fish were adapted to 75% sea water during their smolt stage. High mortality occurred with fish kept in 100% sea water over a prolonged period of time, whereas fish survived indefinitely in 75% sea water (cf. Parry, 1960). No attempt was made to determine the sex of the fish as there is little gonadal development in yearlings. It was assumed that the sex of juvenile trout would not influence their growth rate, as Brown (1946) found no apparent difference in growth of two-year old male and female trout.

During the experimental period the fish were kept in polythene tanks at a density of eight fish per 100 litres. They were separated from each other by clear perforated perspex partitions to allow individual feeding and to prevent injury due to aggressive behavior. At all times fish were fed EWOS No. 4, yearling trout pellets (Locke and Linscott, 1969). Each compartment was individually aerated to ensure maximum oxygenation, since food consumption and growth are known to decline with an appreciable decrease in oxygen level (Hermann and Warren, 1962). Water was recycled through carbon and synthetic cotton filters and completely changed once a week.

Specific growth rates, nitrogen assimilation and excretion rates of salt- and freshwater fish were measured from a period of April to May when the freshwater trout were in smolt condition, and again from July to August when the freshwater trout were post-smolts.

Specific growth rates

Saltwater and freshwater fish were both divided into four groups, each group receiving a different daily ration. Group A was fed *ad libitum*; group B, approximately 0.009 g of food per day per g initial wet weight of fish; group C, 0.0025 g food per day per g wet weight; and group D was starved for the duration of the experiment.

Fish were weighed individually at the beginning and end of the six week feeding period to assess their growth during this time. Prior to the weighings the fish were starved for three days to ensure that their alimentary tracts were empty (Windell and Norris, 1969). At the time of the initial and final weighings several fish were killed by spinal severance, to determine both their nitrogen content and dry weights.

Nitrogen excretion and assimilation

The semi-micro Kjeldahl method (Hawk, Oser and Summerson, 1954) was used for all nitrogen analysis, employing Jacob's (1959) catalyst with concentrated

sulphuric acid and hydrogen peroxide. The ammonia released upon steam distillation was collected in dilute hydrochloric acid and estimated photometrically, at 480 μ after Nesslerization.

To ensure that all fish had the same recent nutritional history, individual fish were fed a 1.5% body weight ration for a ten-day period, prior to measurement of their nitrogen excretion at various times over the succeeding ten-day stravation period. The initial measurement commenced one hour after final feeding. Nitrogen excretion was measured by the increase in nitrogen content of a known volume of water in which the fish were kept for twenty-four hours. These measurements produced a range of nitrogen excretion values for individual fish from fed to starved conditions.

Measurement of nitrogen excretion for the first two days of starvation, when the fish continue to defecate, is somewhat problematical. Faeces encourage bacterial action and the soluble fraction of the faeces increases the nitrogen content of the water. In fresh water, bacterial action in the presence of faeces induces a twenty-five per cent reduction in soluble nitrogen over a twenty-four hour period. No such action was detected either in salt water or in non-contaminated samples. However, no corrective action was taken in freshwater fish, as their experimental feeding regime was effective in restricting defecation to the last three hours of the nitrogen excretion measurement period. Consequntly a maximum four per cent reduction of soluble nitrogen was possible during this time. Approximately 40–60% of the total faecal nitrogen content of individual fish was found to be water soluble. It was therefore necessary to estimate the degree of contamination for each individual fish on the first day of starvation, in order to get a more realistic measurement of their nitrogen excretion. After the twenty-four hour measuring period of the first day, the water in which the fish had been kept was filtered through double layered Whatman G.F.A. 7 cm glass fiber discs to remove the particulate faeces suspended in the water. The nitrogen content of both filtrate (TN) and particulate material (PN) was then measured. The nitrogen content of the particulate faeces was used to estimate the proportion of total soluble nitrogen content of the filtrate that could be attributed to faecal contamination (SN), using the following equation: $SN = Sf/Pf \times PN$, where SN equals the soluble faecal nitrogen contamination, PN equals the measure of nitrogen content of the particulate faeces, and Sf/Pf equals the ratio of soluble to particulate nitrogen in the whole faeces of individual fish, collected at the time of the assimilation experiments described later. Thus the corrected nitrogen excretion (EN) for day one, allowing for faecal contamination is $EN = TN - SN$, where TN is the total soluble nitrogen content of the filtrate.

The gut assimilation rates of individual fish were measured following the nitrogen excretion measurements, as the direct faecal collection caused some scale loss, and resulted in a certain amount of ionic and osmotic stress which may have adversely affected the nitrogen excretion results. On the tenth day of starvation, feeding was recommenced for a further seven days with a 1.5% body weight ration containing 1% chromic oxide. The chromic oxide acts as an indigestible non-absorbable substance to give a baseline from which assimilation can be measured. At the end of the feeding period faecal samples were collected directly from the fish by applying slight pressure with thumb and forefinger between the ventral fin and anus. Care was taken to ensure faecal samples were not contaminated with

urine. The samples were dried at 75° C for two weeks and sub-samples were then taken for determination of nitrogen and chromic oxide content. The diet was analyzed for the same parameters. Chromic oxide content was determined by wet ashing with concentrated nitric acid and 60% perchloric acid and analyzed photometrically at 350 μ (Furukawa and Tsukahara, 1966). The apparent digestibility of assimilation was calculated from the formula of Maynard and Loosli (1962): digestibility $=100 - 100 \times (Cr_2O_3$ in diet/Cr_2O_3 in faeces) $\times$ (Nitrogen in faeces/Nitrogen in food).

Data compilation and analysis

Growth rate. Under controlled constant laboratory conditions growth is a multiplicative process which in the early stages of life follows an exponential curve (Brett, Shelbourn and Hoop, 1969). Growth was therefore expressed as specific growth rate, the instantaneous rate of gain in weight per unit weight: specific growth rate $= (\ln W_2 - \ln W_1/t_1 - t_2) \times 100\%$, where $\ln W_1$ and $\ln W_2$ are the natural logarithms of the weight of individual fish at time, t_1 and t_2 respectively, t being expressed in days.

The relationship between specific growth rates and food consumption is presented graphically; consumption being calculated as mean per cent ration, based on the initial (w_1) and final (w_2) weight: mean per cent ration = [daily ration (g)/$(w_1 + w_2/2)$] $\times$ 100%.

In addition, the effectiveness with which fish are able to convert food into flesh is numerically expressed in the two forms of gross and net growth efficiency. Gross efficiency (K) is the ratio of specific growth rate (ΔB) to consumed food (C), whereas the net efficiency (N) is the ratio of specific growth rate (ΔB) to the ration consumed in excess of maintenance ration ($C - M$).

Nitrogen metabolism. The nitrogen metabolism of a feeding fish can be expressed as: Consumed N − Faecal N = Assimilated N = Retained N + Excreted N. This amount of nitrogen retained for growth is calculated from the measure consumption, assimilation and excretion rates.

Knowing the nitrogen content of the fish flesh, it is possible to calculate the nitrogen retention directly from measurements of specific growth rate values at the same ration.

Two numerical expressions of protein utilization with respect to growth in trout are obtained using the equations: (1), the efficiency of protein utilization for growth = (Protein N retained/Protein N absorbed) $\times$ 100% and (2), the gross efficiency = (N retention/N consumption) $\times$ 100%.

Results

Specific growth rates

Specific growth rates relative to food consumption are very similar in saltwater and freshwater fish when measured from April to May (Table I, Fig. 1). Maintenance rations are identical, and gross and net efficiencies at maximum ration show no significant difference (Table II). However, the fresh water fish have a tendency to consume a greater amount when fed *ad libitum*, leading to a slightly higher

TABLE I

Statistical parameters of the specific growth results.

Mean ration level	Mean specific growth rate ΔB	Sample size n	Standard deviation	Standard error of the mean (s.e.m.)
Salt water, spring (Fig. 1)				
A. 1.385%	0.814%	8	0.148	0.052
B. 0.752%	0.535%	6	0.122	0.050
C. 0.238%	0.009%	8	0.084	0.030
D. 0	−0.459%	7	0.075	0.028
Fresh water, spring (Fig. 1)				
A. 1.708%	0.941%	8	0.143	0.051
B. 0.829%	0.583%	8	0.069	0.024
C. 0.251%	0.011%	8	0.046	0.016
D. 0	−0.415%	8	0.032	0.009
Salt water, late summer (Fig. 2)				
A. 1.412%	0.789%*	10	0.156	0.049
B. 0.668%	0.356%	7	0.115	0.044
C. 0.229%	−0.106%	7	0.068	0.026
D. 0	−0.434%	9	0.136	0.048
Fresh water, late summer (Fig. 2)				
A. 1.665%	0.587%*	11	0.154	0.046
B. 0.665%	0.410%	5	0.100	0.045
C. 0.220%	−0.051%	6	0.053	0.021
D. 0	−0.381%	7	0.162	0.061

* Test for significant difference between mean specific growth rates of seawater and freshwater summer fish at ration A. $t = 2.84, f = 19, P = >0.01$.

specific growth rate at this ration. In contrast, in July and August the specific growth rate of the freshwater fish is significantly lower ($P > 0.01$) at *ad libitum* ration (Table I, Fig. 2). Consequently, during this period, both gross and net efficiencies are higher in the *ad libitum* saltwater fish (Table II). No such differences are apparent at the lower ration sizes. Body water content is not a contributive factor to the growth rate difference at *ad libitum*, as there is no difference in the dry weight of saltwater and freshwater fish (Table II).

Nitrogen metabolism

The nitrogen excretion rates of both the freshwater and saltwater trout decline at a similar rate over the initial six days of the starvation period after which the rate of excretion stabilizes at a more basal level (Figs. 3 and 4). (All excretion values for day 1 have been corrected for faecal contamination.) However, the actual nitrogen excretion rates of the post-smolt freshwater fish (July to August) differs

TABLE II

Growth parameters of freshwater and saltwater trout.

	Specific growth rate at *ad libitum* ration ΔB	*Ad libitum* ration C per cent mean ration	Nitrogen input mg N/kg fish/day	Gross efficiency at *ad libitum* ration $K = \Delta B/C$	Maintenance ration M	Net efficiency at *ad libitum* ration $N = \Delta B/C - M$	Dry weight as % wet weight	Nitrogen content mg N/gm dry wt fish at *ad libitum* ration
Salt water, April to May	0.814%	1.38	533	0.59	0.25%	0.72	22.2%	111
Fresh water, April to May	0.941%	1.70	656	0.55	0.25%	0.65	22.9%	110
Salt water, July to August	0.789%	1.41	544	0.56	0.27%	0.69	21.7%	110
Fresh water, July to August	0.587%	1.66	642	0.35	0.25%	0.42	21.0%	108

significantly from those of the saltwater fish and freshwater smolts (April to May) in two respects. Initially, the total daily nitrogen excretion of the post-smolt is higher, yet by the time nitrogen excretion has reached basal level, the post-smolt excretion rate is lower than that of the other fish (Table III).

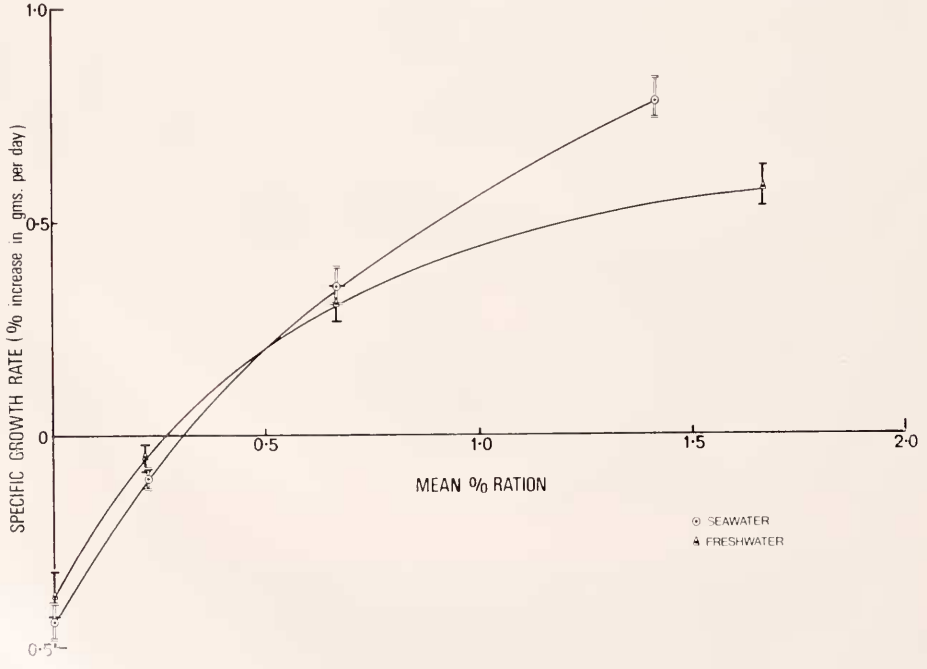

FIGURE 1. The relationship between specific growth rate and food consumption at 12° C for yearling rainbow trout during April to May.

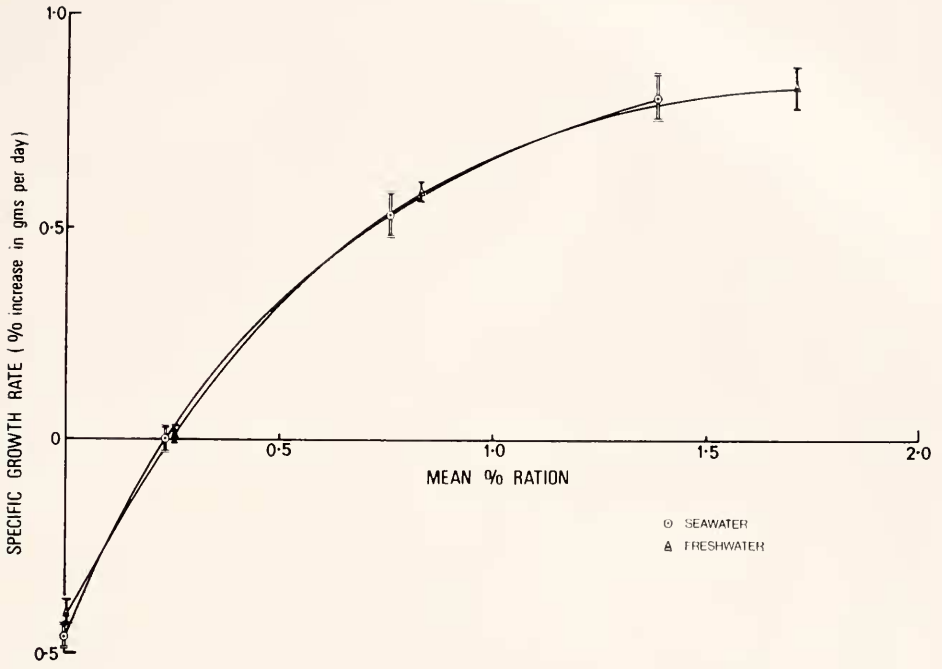

FIGURE 2. The relationship between specific growth rate and food consumption at 12° C for yearling rainbow trout during July to August.

Nitrogen assimilation

No differences are apparent in the gut assimilation of the freshwater smolt, post-smolt and saltwater fish. Mean assimilation rates were 77.1% in salt water and 78.2% in fresh water. Faeces inevitably include metabolic faecal nitrogen secreted

TABLE III

The nitrogen excretion on the first and tenth day of starvation of fed freshwater and saltwater trout.

	Number of fish	Day 1 nitrogen excretion (mg N/kg wet wt.) ($\bar{X} \pm$ s.e.m.)	s.d.	*P* value compared with freshwater post-smolt	Day 10 basal nitrogen excretion (mg N/kg wet wt.) ($\bar{X} \pm$ s.e.m.)	s.d.	*P* value compared with freshwater post-smolt
Fresh water post-smolt, July to August	21	363 ± 13.6	62.5	—	60 ± 3	14	—
Fresh water smolt, April to May	13	316 ± 13.5	48.8	0.025	92 ± 5	17	0.001
Salt water, April to May	14	286 ± 10.8	41.8	0.001	97 ± 6	25	0.001
Salt water, July to August	12	287 ± 6.8	23.7	0.001	93 ± 9	30	0.001

s.d. = standard deviation; $\bar{X} \pm$ s.e.m. = mean ± standard error of the mean.

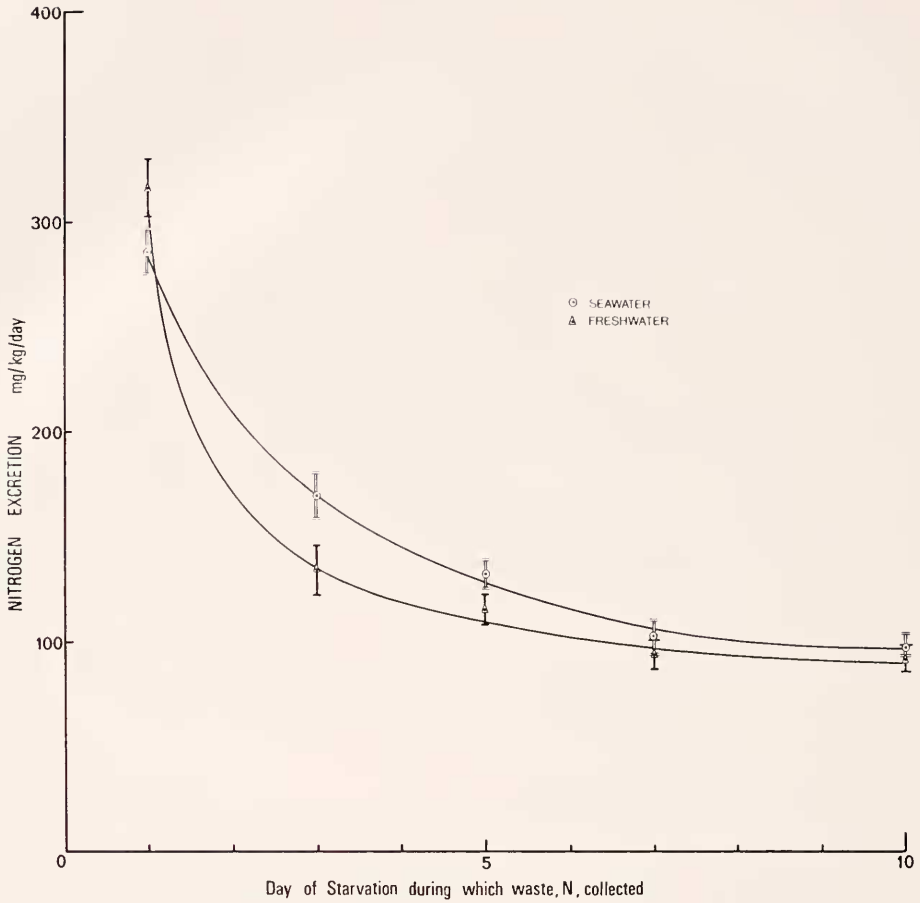

FIGURE 3. The nitrogen excretion of freshwater smolt and saltwater fish (April to May) over an initial ten day starvation period.

into the gut, which should not be considered as non-assimilated dietary nitrogen. Nose (1967) estimated that metabolic faecal nitrogen in rainbow trout amounts to 50–150 mg N/100 g of diet, which represents 5–17% of the total faecal nitrogen in

TABLE IV

The nitrogen retention efficiencies of freshwater and saltwater trout.

	Gross efficiency from nitrogen excretion	Efficiency of protein utilization	Gross efficiency from specific growth rates
Fresh water post-smolt, July to August	15.4%	19.7%	21%
Fresh water smolt April to May	23.4%	30.1%	36%
Salt water, April to May	27.3%	35.6%	33%
Salt water July to August	27.0%	35.4%	35%

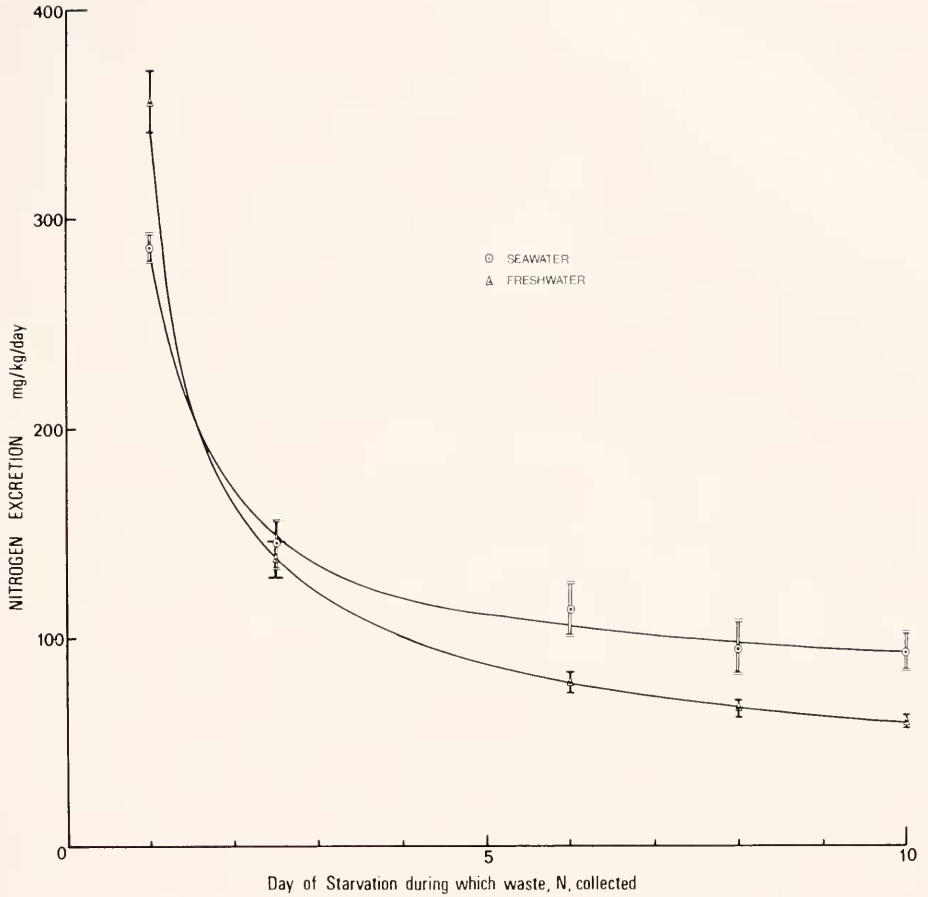

FIGURE 4. The nitrogen excretion of freshwater post-smolt and saltwater fish (July to August) over an initial ten day starvation period.

the present experiments. Taking the larger factor into account, the assimilation of the freshwater fish could be as high as 81.9% for the freshwater fish and 81% for the saltwater fish. This correction increases the proportion of excreted nitrogen by an amount equivalent to the increased assimilation. Values for nitrogen retention in the nitrogen balance equation are unaffected.

Nitrogen balance

A 1.5% ration gives a trophic input of 578 mg N/kg wet weight of fish/day, of which 452 mg N are assimilated in the freshwater fish and 446 mg N in the saltwater fish. If the nitrogen excretion values on the first day of starvation (Table III) are taken as representative of the nitrogen loss of a fully fed fish, then the difference between assimilation and excretion and the nitrogen retained for protein elaboration or growth is: 89 mg N/kg in the post-smolt freshwater fish (July to August), 136

mg N/kg in the smolt freshwater fish (April to May), 159 mg N/kg in the saltwater fish (July to August), 159 mg N/kg in the saltwater fish (April to May). Comparison between retention efficiencies based on excretion and specific growth rates (Table IV) show a discrepancy in both freshwater and saltwater fish. This could have arisen from the differences in experimental approach, or, more likely, from the difference in size distribution of the fish used in the excretion and growth experiments (40–60 g and 30–40 g, respectively), as Gerkin (1971) and Pandian (1967) have both reported decreased efficiency in protein utilization with increase in size in *Lepomis macrochirus* and *Megalops cyprinoides*. This discrepancy does not alter the basic conclusion that the lower specific growth rate of the fresh water post-smolt at near maximum ration, compared to that of the smolt and saltwater fish, results from a decreased efficiency in nitrogen retention and not from differences in food consumption or assimilation. However, the lower basal excretion rate of the post-smolt (Table III) indicates an increased efficiency in conservation of body protein under the catabolic conditions of starvation.

Discussion

Previous reports on the relationship of growth to nitrogen metabolism in freshwater trout have given similar assimilation values, from 99.5% for milk caesin to 83.9% for egg albumin (Nose, 1967) and 80–90% for *ad libitum* egg white and liver (Atherton and Aitken, 1970). Although the physiological state of these fish was not given, their specific growth rate is comparable to the 0.94% rate of the present freshwater smolt as opposed to the lower 0.59% growth rate of freshwater post-smolt, both of which assimilated 78–81% of their 642–656 mg N/kg fish/day *ad libitum* ration. The seasonal change in *ad libitum* growth rate in freshwater trout under natural and constant temperature conditions has been previously described by Swift (1956) and Brown (1946). Brown (1946) attributed this change to an annual endogenous growth rate cycle, composed of an autumnal check, followed by rapid spring growth, slow summer growth, and finally another autumnal check. Swift (1961) correlated the cycle with seasonal environmental temperature change, which suggests a possible synchronization of the endogenous cycle with environmental temperature change. The present results show a close association between high specific growth rates and saltwater tolerance in trout. In fresh water the rapid spring growth of the cycle is coincident with the euryhalinity of the smolt phase, whereas the slower summer growth rate occurs at the time when the freshwater trout are in a stenohaline post-smolt condition. No such change in growth rate is apparent in the saltwater fish which continue to grow at an elevated rate throughout the year.

Under the same immediate experimental conditions and feeding regime, higher growth rates result directly from increased ability to retain assimilated nitrogen, and not from increased food availability, consumption or assimilation. The reciprocal nature of nitrogen excretion to specific growth rate in fully fed fish is seen in the seasonal fluctuation of excretion levels in fresh water and the continuous low rate of excretion in salt water, indicating an increased protein anabolic capacity in the fully fed freshwater smolt and saltwater fish. The basal nitrogen excretion of starved fish also show a seasonal fluctuation associated with the euryhalinity-stenohalinity cycle in fresh water which is not apparent in salt water.

However, in contrast to fully fed fish, the basal nitrogen excretion of starved fish suggests an increased protein catabolism in the freshwater smolt and salt water fish; post-smolt fish having a lower demand on their protein reserves under starvation conditions. The increased rates of synthesis in fed fish and degradation in starved fish may represent an overall increase in protein turnover that under maximum trophic input leads to higher specific growth rates.

Brown (1946) postulated the increased spring growth rate in the freshwater trout was endogenously induced by variation in the activity of the endocrine system. The close association between the increased protein metabolism and saltwater tolerance suggests that physiological induction of euryhalinity in freshwater fish simultaneously stimulates protein turnover and therefore growth. If the smolts remain in fresh waters past the migratory period, their return to stenohalinity is concomitant with decreased protein turnover. However, if the smolts do migrate to the sea, their ionic regulatory system is activated, tolerance to salt water maintained, and protein turnover stimulated further.

Smolt transformation and salt water tolerance are accompanied and most likely induced by thyroid, intrarenal and somatotrophic activity (Baggerman, 1960; Maetz, 1968; Olivereau, 1954). The hormonal induction and control of saltwater tolerance may possibly be the endogenous activity required to produce the annual growth rate cycle in freshwater and the sustained growth in saltwater *S. gairdneri*.

Summary

If growth is considered over the complete annual cycle, saltwater trout grow at a greater rate than freshwater trout when fed *ad libitum*. However, during the spring, the growth rate of the freshwater smolt increases to a level equivalent to that of the saltwater fish. This is not primarily a result of differences in temperature of the natural seawater and freshwater environments, nor is it associated with differences in food quality, consumption or assimilation rates, but results from the increased rates of protein synthesis and degradation found in freshwater smolt and saltwater fish. The close association of the increased rates of protein metabolism with the smoltification process in freshwater fish, the continuation of this increase in the saltwater fish and the decrease found in the post-smolts remaining in fresh water, all suggest that the processes which bring about the transformation of a stenohaline freshwater trout to a euryhaline trout increase its rates of protein metabolism. It is known that various hormones play a role in the salinity adaptation of the migratory salmonids. Thus the possibility exists that such hormonal activity, primarily linked with osmotic and ionic regulation, may have a secondary effect on the protein metabolism and specific growth rate of the migratory rainbow trout.

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